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Survival of Hepatitis A Virus in Feces After Drying and Storage for 1 Month

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Hepatitis A virus in feces remained viable after being dried and then stored at 25°C and 42% relative humidity for 30 days, as evidenced by infection of two marmosets inoculated with 1 ml of the treated fecal material. The virus was excreted in stool specimens from these animals, and seroconversion to antibody to hepatitis A virus occurred 28 and 35 days post-inoculation.

In a recent study (2), we showed that hepatitis A virus (HAV) antigen in an 18% (wt/vol) stool suspension remained immunologically reactive after being stored for 15 days at 25°C and 42% relative humidity in both the liquid and dried states. These results, however, did not indicate the extent of inactivation or survival of HAV after similar treatment. To determine whether HAV is still infectious after being dried and stored for up to 1 month, we inoculated susceptible marmosets (*Saguinus mystax*) with the treated stool suspension. This study is part of an ongoing series of experiments to determine the resistance of HAV to a variety of physical and chemical agents.

A single-source inoculum (designated HLD-2) was obtained from a chimpanzee (no. 905) that had been infected with an 18% (wt/vol) stool suspension from a human case of acute hepatitis A. HAV was detected by solid-phase radioimmunoassay (SPRIA) (7) in feces collected from this chimpanzee 6 days after inoculation. The specificity of SPRIA for the detection of HAV antigen was determined by testing each specimen on a microtiter plate coated with either anti-HAV immunoglobulin G (IgG) or normal IgG (3). The P/N ratio for each specimen was calculated by dividing the counts per minute from the anti-HAV-coated plate (P) by the corresponding counts per minute from the normal IgG-coated plate (N). Significant elevations in alanine aminotransferase activity were recorded on days 15 to 33, and immunofluorescent-antibody testing of liver biopsy tissue showed it to be positive for HAV. Seroconversion to antibody to HAV (HAVAB, Abbott Laboratories) occurred at 12 days post-inoculation. An 18% (wt/vol) suspension of a fecal specimen taken within 8 days of inoculation from chimpanzee no. 905 was prepared by adding 2 g of feces to 10 ml of 0.85% NaCl with 3-mm glass beads and blending the solution with a Vortex mixer for 2 min. The suspension was clarified at 4,800 × *g* for 20 min; the supernatant fluid dispensed in 0.1-ml amounts into ampoules which were flame sealed and stored at −40°C. The HLD-2 inoculum was shown to be positive for HAV antigen by SPRIA, and HAV particles were seen by immune electron microscopy.

The first set of experiments was done by inoculating three *S. mystax* marmosets (nos. 00-17, 00-24, and 00-38) with HLD-2 that had been dried and stored for 7 days. Four 0.1-ml samples of HLD-2 were thawed, transferred to 1-dram (ca. 4-ml) screw-cap glass vials, and dried for 16 h at 25°C under vacuum over anhydrous calcium sulfate. When dry, the vials were loosely capped and placed at 25°C in a desiccator jar over a saturated aqueous solution of potassium carbonate, which created an enclosed relative humidity of 42% (1). After 7 days, each vial was rehydrated with 1 ml of normal saline. The rehydrated material was pooled, and each of the three marmosets was inoculated intravenously with 1 ml of the pooled material. The animals were monitored twice weekly for elevated serum isocitrate dehydrogenase (SICD; Sigma Chemical Co.) activity and seroconversion to anti-HAV. Liver biopsies were obtained when SICD activity was elevated; feces were collected daily for HAV testing by SPRIA.

After being dried and stored for 1 week at 25°C and 42% relative humidity, HLD-2 was still infectious (Table 1). Shedding of HAV into the feces was detected as early as 8 days after inoculation, and HAV was detected by immunofluorescent assay in liver biopsies within 25 days. Elevations in SICD activity and serocon-

TABLE 1. Infectivity of HLD-2 that had been dried and stored for 7 days at 25°C and 42% relative humidity

Marmoset no.	First positive result (days post-inoculation) for test:			
	SPRIA[a]	SICD	Anti-HAV IgM	Immunofluorescent antibody[b]
00–24	8	14	17	25
00–38	10	21	21	25
00–17	17	31	32	25

[a] Stool specimens were tested for HAV.
[b] Test was performed on liver biopsy material.

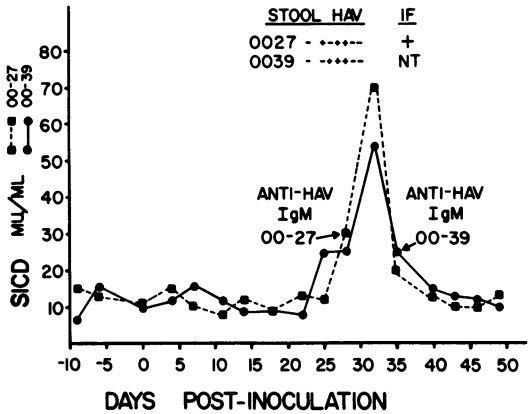

FIG. 1. Course of HAV infection in marmosets after inoculation with HLD-2 that had been dried and stored for 30 days at 25°C and 42% relative humidity.

version to anti-HAV occurred within 32 days post-inoculation.

Since drying and storage for 7 days did not affect the infectivity of HAV in this stool suspension, four additional 0.1-ml volumes of HLD-2 were thawed and dried as in the previous experiment. These specimens were then stored for 30 days at 25°C and 42% relative humidity. The contents of each vial were then rehydrated and pooled as previously described, and 1 ml of the pooled material was inoculated into each of two *S. mystax* marmosets (nos. 00-27 and 00-39). Serological monitoring for HAV infection was the same as for the 7-day experiment.

The course of HAV infection in marmosets 00-27 and 00-39 after the inoculum had been dried and stored for 30 days is shown in Fig. 1. The animals showed marked increases in SICD levels at 28 and 35 days post-inoculation. HAV antigen was detected in fecal specimens, and seroconversion to anti-HAV IgM (HAVAB-M, Abbott Laboratories) occurred within 35 days of inoculation.

These results indicate that HAV will remain viable in feces and cause infection after being dried and stored for at least 30 days under conditions simulating a typical environmental exposure. It is reasonable to assume that in actual contamination situations similar material should be considered a primary infection hazard. The data presented support our past recommendations for immediate and meticulous cleaning and disinfection of potentially contaminated surfaces, particularly in high-risk environments such as those found in day-care centers (5, 6), institutions, or clinical laboratories where stool specimens are routinely examined. Since the chemical resistance spectrum of HAV is still unknown, we recommend the use of germicidal chemicals having at least an intermediate level of action (e.g., sodium hypochlorite or certain iodophors) (4).

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